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Serotyping and Ribotyping of *Salmonella* Using Restriction Enzyme *Pvu*II

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ABSTRACT

The subtyping and identification of bacterial pathogens throughout food processing and production chains is useful to the new hazard analysis critical control point–based food safety plans. Traditional manual serotyping remains the primary means of subtyping *Salmonella* isolates. Molecular biology techniques, however, offer the promise of more rapid and sensitive subtyping of *Salmonella*. This study evaluates the potential of restriction enzyme *Pvu*II, followed by probing with the rRNA operon from *Escherichia coli,* to generate serotype-specific DNA fingerprints. A total of 32 identified serotypes were found with an overall agreement in 208 of the 259 (80%) isolates tested between U.S. Department of Agriculture serotype identification and riboprint serotype identification. Many of the isolates that did not correlate were serotype identified as *Salmonella* Montevideo, which indicates that for this serotype, there are multiple ribotypes. When *Salmonella* Montevideo isolates were not included, the ribotype identification agreed with serotyping in 207 of the 231 (90%) isolates. The primary outcome of any ribotyping procedure is to give distinct ribotype patterns. This extensive poultry epidemiological study demonstrates that, in addition to ribotype patterns, the identification of isolates to known serotypes provides the investigator with additional information that can be more useful than traditional epidemiology and isolate identification studies.

The subtyping and identification of bacterial pathogens throughout food processing and production chains is very important to hazard analysis critical control point (HACCP)–based food safety plans. Poultry production facilities are often contaminated with *Salmonella,* and rapid identification of these isolates is key to quickly finding and eliminating the source as well as determining the extent of contamination. Traditional serotyping remains the primary means of subtyping *Salmonella* isolates. Molecular biology techniques, however, offer the promise of more rapid and sensitive subtyping of *Salmonella.* The automated RiboPrinter microbial characterization system (Qualicon, Inc., Wilmington, Del.) was evaluated in this study to determine its efficacy for accurately assigning serotype names using a *Salmonella* set isolated from poultry.

Previous studies have shown ribotyping often correlates with serotyping and therefore offers a more rapid method of subtyping *Salmonella (5, 6).* The data generated previously relied on digestion of the bacterial genome by *Eco*RI and subsequent hybridization probing using the rRNA operon from *Escherichia coli.* The restriction enzyme chosen to digest the bacterial genome will affect the number and position of bands found in the RiboPrinter data formed *(8, 11).* Dambaugh et al. *(4)* presented evidence suggesting that the ribotyping of *Salmonella* using the restriction enzyme *Pvu*II increased the incidence of discreet ribotype patterns for the most common *Salmonella* serovars. This study evaluates the potential of *Pvu*II to generate serotype-specific DNA fingerprints.

Recent unpublished data have also shown that among the most common serovars, *Pvu*II digestions instead of *Eco*RI result in a greater number of serotype-specific patterns among many of the most common serotypes *(1).* In this study, the ribotype patterns generated were compared to a 260-*Salmonella* pattern database generated using restriction enzyme *Pvu*II by Qualicon. The database was constructed primarily of type strains and clinical strains. The isolates used in this study, however, were all isolated from poultry. Many of the same serotypes predominant among human clinical isolates are also common among poultry isolates. *Salmonella* Typhimurium and *Salmonella* Enteritidis, for example, account for approximately 50% of all human isolates and are also two of the most frequently identified poultry isolates.

MATERIALS AND METHODS

Bacterial strains. This study used 259 *Salmonella* isolates consisting of 32 unique serotypes or subtypes (Table 1) isolated from a variety of poultry sources, including poultry feces, carcass rinses, scald water, transport coop, paper pads, water cups, litter/bedding, cecal contents, dust/dirt, fly samples, drag swabs, post-transport coop, boot swab, postscald water, and water lines. Serotyping of the isolates was done using traditional manual methods at the National Veterinary Services Laboratory (Ames, Iowa). The isolates were all collected from four separate growers on four separate grow-out periods over a 1-year period in 1998. The isolates were stored on protective beads at −80°C and grown on brain heart infusion broth (Qualicon). Twenty-three serotypes had more than one isolate for comparison, while nine serotypes had only one isolate to test (Table 1).

Ribotyping. Ribotyping was performed using the automated RiboPrinter microbial characterization system (Qualicon). The standard protocols prescribed by the manufacturer were followed

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TABLE 1. *Comparison of RiboPrinter identification to conventional serotyping*

USDA *Salmonella* serotypes	*n*	RiboPrinter *Salmonella* serotypes	USDA/RiboPrinter agreement
S. 4,5,12 I-monophasic	20	*S.* Typhimurium	20/20
S. Agona	7	*S.* Agona (4), Abaetetuba (1), none (2)	4/7
S. Bareilly	1	*S.* Bareilly	1/1
S. Braenderup	1	*S.* Chicago/Virchow	0/1
S. Brandenburg	9	*S.* Reading_Brandenburg	9/9
S. Bredeney	1	*S.* Bredeney	1/1
S. Derby	3	*S.* Derby	3/3
S. Enteritidis	23	*S.* Enteritidis	23/23
S. Give	1	*S.* Give	1/1
S. Hadar	6	*S.* Hadar	6/6
S. Havana	1	*S.* Drypool	0/1
S. Heidelberg	10	*S.* Heidelberg (9)/none (1)	9/10
S. Infantis	13	*S.* Infantis Newport (12), Thompson (1)	12/13
S. Inverness	2	*S.* Mississippi_Minnesota (1), none (1)	0/2
S. Johannesburg	1	*S.* Johannesburg_Mbandaka	1/1
S. Kentucky	11	*S.* Kentucky	11/11
S. Lille	1	None (1)	0/1
S. Livingstone	3	*S.* Livingstone (2), none (1)	2/3
S. Mbandaka	14	*S.* Mbandaka	14/14
S. Molade	6	*S.* Lille (3)/S. Javiana (2), Kentucky (1)	0/6
S. Montevideo	28	*S.* Montevideo (1), S. Reading (9), none (18)	1/28
S. Muenchen	1	*S.* Hadar	0/1
S. Newington	1	*S.* Anatum_Newington	1/1
S. Newport	4	*S.* Newport_Bardo	4/4
S. Ohio	5	*S.* Ohio	5/5
S. Ouakum	2	*S.* Muenchen	0/2
S. Schwarzengrund	19	*S.* Schwarzengrund (18), Thompson (1)	18/19
S. Senftenberg	10	*S.* Senftenberg (9), Kentucky (1)	9/10
S. Tennessee	2	*S.* Javiana_Paratyphi (1), none (1)	0/2
S. Thompson	27	*S.* Thompson	27/27
S. Typhimurium	22	*S.* Typhimurium	22/22
S. Typhimurium (Copenhagen)	4	*S.* Typhimurium (Copenhagen)	4/4

(3, 7). Standard reagents were used in all steps of the analysis. This method involves the lysis of target cells with the release of cellular DNA, *Pvu*II digestion of the chromosomal DNA, and separation of the resulting fragments by agarose gel electrophoresis, followed by automated Southern blot hybridization probing with the rRNA operon from *E. coli (9)* as a chemiluminescent probe. Images were acquired with a charge-coupled-device camera and processed by RiboPrinter system software. The software normalizes fragment pattern data for band intensity and relative band position as opposed to molecular-weight markers, which are incorporated into the standard reagent package. Genetic fingerprint patterns were identified by matching these patterns ($\geq$0.85) with those supplied in a *Salmonella Pvu*II database. All isolates were also clustered into groups according to their similarity. Groups were formed and populated by isolates with 90.0 to 93.0% homology.

RESULTS AND DISCUSSION

Thirty-two serotypes were identified in this national poultry epidemiology study. For 16 of these 32 serotypes, there was a 100% correlation between the serotype as identified by the U.S. Department of Agriculture (USDA) and the serotype as identified by the *Pvu*II riboprint, and there was an overall agreement in 208 of the 259 (80%) isolates tested between USDA-serotyped identification and riboprint serotype identification (Table 1). Twenty-seven of the 51 isolates that did not agree between the two systems were from the USDA-identified *Salmonella* Montevideo group. This suggests that there are multiple ribotypes of *Salmonella* Montevideo. When *Salmonella* Montevideo isolates were not included, the ribotype identification agreed with serotyping in 207 of the 231 (90%) isolates. Other serotypes that exhibited poor correlation between serotyping and ribotyping included *Salmonella* Agona, *Salmonella* Inverness, *Salmonella* Ouakum, and *Salmonella* Tennessee. Other serotypes that gave multiple ribotype identifications included *Salmonella* 4,5,12 I-monophasic Typhimurium, *Salmonella* Heidelberg, *Salmonella* Schwarzengrund, and *Salmonella* Typhimurium.

The nomenclature of the *Salmonella* group has progressed through a succession of taxonomical schemes based on biochemical and serological characteristics and on principles of numerical taxonomy and DNA homology. The Kauffmann-White scheme was the first widely accepted systematic classification system *(10).* According to the World Health Organization's Collaborating Center for Ref-

erence and Research on *Salmonella* (Institute Pasteur, Paris, France), there are 2,463 currently recognized serovars of *Salmonella (2).* This study examined only 32 of these serovars, but these account for 13 of the top 15 non-human isolates reported to the USDA in 1997 and for 10 of the top 15 human isolates reported to the Centers for Disease Control in 1998.

The automated RiboPrinter system offers the advantages of speed and simplicity of operation. This study was designed to determine the usefulness and efficacy of the RiboPrinter system to subtype and serotype *Salmonella* isolates originating from poultry. Eighty to 90% correlation between both serotypes and isolates from this study indicates that the RiboPrinter could be added to manual serotyping as a rapid and reliable *Salmonella* subtyping method. Because the database of the system can be updated with every isolate that is tested, it is only reasonable to assume that, over time, the number of isolates and riboprint patterns in the database would expand and that the correlations between serotyping and riboprint patterns would become even stronger. In general, although some isolates did not correlate directly with a manual serotype, there was a trade-off in the degree of discrimination offered by the technology. Some of the serotypes had multiple ribotypes, thereby providing more discrimination than serotyping, while for other isolates, there were multiple serotypes identified as common serotypes. By exploiting the relationship between RiboPrinter system data and serotype identification, scientists can take advantage of the automated high throughput capability of the instrument while generating historically relevant ribotype and serotype data.

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